EFFECT OF STROMAL FIBROBLASTS ON ANTIBODY FORMATION IN NONADHERENT SPLEEN CULTURES

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Stromal fibroblasts from monolayer cultures of thymus, spleen, and bone marrow cells, when added to nonadherent suspension cultures of spleen cells have a significant influence on the accumulation of antibody-forming cells (AFC). Stromal fibroblasts of thymic origin stimulate AFC formation, whereas those of bone-marrow origin inhibit antibody formation in these cultures. Stromal fibroblasts of splenic origin have the same effect on AFC formation as adherent cells. Stromal fibroblasts irradiated in a dose of 5000 R have the same action as unirradiated cells. The action of stromal fibroblasts on antibody formation in culture is manifested only if they adhere to the surface of the culture vessel.

KEY WORDS: stromal fibroblasts; nonadherent cells; antibody formation in vitro.

As has been shown previously, stromal mechanocytes of hematopoietic organs have a significant effect on antibody forming cell (AFC) production in vitro. This action clearly depends on the dose and its direction depends on the origin of the mechanocytes added.

The object of this investigation was to study whether the action of stromal mechanocytes is mediated through nonlymphoid A cells, the presence of which is required for AFC formation in vitro; whether adhesion and proliferation of the fibroblasts are necessary for their action on AFC production; and what stage of AFC development is most sensitive to the action of stromal mechanocytes.

The plan of the investigation was to remove A cells from a suspension of spleen cells and replace them by stromal fibroblasts isolated from different organs; to use fibroblasts irradiated in a dose of 5000 R; to add fibroblasts at different stages of culture.

Culture vessels with a special antiadhesive covering, preventing adhesion of fibroblasts, were used in some experiments.

EXPERIMENTAL METHOD

Cell lines of stromal fibroblasts were isolated from the spleen, thymus, and bone marrow of rabbits by the method described previously [1]. The stromal fibroblasts were used after 3-6 subcultures. The method of Mishell and Dutton [4] was used to induce antibody synthesis in vitro. The A cells were removed by Mosier's method [5], by triple adhesion on glass or plastic. Cells remaining nonadherent after the 3rd adhesion were regarded as A-deficient. Cultures were grown in Millipore plastic dishes 50 mm in diameter. In some experiments dishes with an antiadhesive covering were used. The cell density for culture of the complete population was $1.3 \times 10^6/cm^2$ and for culture of nonadherent cells $1.04 \times 10^6/cm^2$. As the antigen, 0.1 ml of a 2% suspension of sheep's red blood cells was added. In some experiments stromal fibroblasts obtained from one donor were used and were added to the same suspension of nonadherent cells. Stromal fibroblasts taken from monolayer cultures were added to the system in a volume of 0.2 ml. Culture was carried out at 37°C in an atmosphere containing 7% O_2, 10% CO_2, and 83% N_2, and the dishes were constantly rotated (9 rpm). The number of AFC was determined on the 4th day of culture by Jerne and Nordin's method of local hemolysis in gel [3]. For each group the number of the living cells was determined by the trypan blue exclusion test and the number of AFC per culture was calculated. The significance of differences was assessed by Student's criterion. The cell suspensions were irradiated at 4°C, from an ÉKU-50 cobalt source with a dose rate of 166 rad/min.

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TABLE 1. AFC Formation in Cultures of Spleen Cells Deficient in Endogenous A-Cells after Addition of Stromal Fibroblasts of Varied Origin

Type of fibroblasts	Inhibition after adhesion, %	No. of fibroblasts added to culture ($\times 10^6$)	AFC per culture, %	Survival rate
Bone-marrow	0	—	100,0±12,26	1,0
		0,1	40,2±7,42	1,0
		0,2	14,6±3,52	0,57
		0,4	24,9±4,96	0,94
	79,94	—	100,0±6,73	1,0
		0,08	13,42±13,42	0,95
		0,16	0	0,89
		0,32	6,62±6,62	0,79
Thymic	0	—	100,0±6,73	1,0
		0,003	136,45±17,01	0,89
		0,012	203,84±58,88	1,11
		0,05	564,2±22,5	1,22
		0,2	256,22±35,75	0,89
		0,4	156,02±41,22	1,42
		0,8	169,0±32,7	1,11
	98,24	—	100,0±0	1,0
		0,012	2437,34±0	1,44
		0,025	5612,92±246,08	1,44
		0,05	3409,94±1460,84	1,34
		0,1	1460,94±0	0,9
Splenic	0	—	100,0±10,04	1,0
		0,005	128,3±4,51	0,97
		0,01	115,86±37,11	1,2
		0,1	50,22±10,04	0,58
		0,2	52,22±4,04	0,75
	79,9	—	100,0±3,33	1,0
		0,005	111,3±22,36	1,07
		0,01	233,54±11,04	1,07
		0,05	350,44±38,9	1,43
		0,1	77,75±11,4	0,89
		0,2	66,75±22,75	1,07

*Survival rate expressed as ratio or number of living cells in cultures with added fibroblasts to number of living cells in control cultures (without added fibroblasts).

EXPERIMENTAL RESULTS

Removal of the A cells from the population of spleen cells led to sharp inhibition of antibody formation compared with the whole population. The effect of stromal fibroblasts on antibody formation in cultures of spleen cells, subjected or not to previous adhesion, is shown in Table 1.

The inhibitory activity of fibroblasts of bone-marrow origin was manifested during association both with the whole population and with the population deficient in endogenous A cells; in the latter case, moreover, inhibition was more marked. The action of fibroblasts of thymic origin also was more marked on splenic cells deficient in endogenous A cells; on the addition of 2.5×10^4 thymic fibroblasts to cultures of nonadherent cells the number of AFC was increased by 56 times, whereas on the addition of the same number of fibroblasts to the complete population of splenic cells the number of AFC was increased by only 2-2.5 times. The effects of addition of splenic fibroblasts to cultures of nonadherent spleen cells, compared with its effect on cultures of the complete population differed not only in intensity, but also in direction. Stromal mechanocytes of splenic origin in this case acted like A cells: small numbers of fibroblasts stimulated antibody formation, whereas large doses led to marked inhibition. In experiments in which splenic cells, freed from endogenous A cells, were associated with fibroblasts of different origin, but grown from tissues of the same donor, the same differences were observed between the action of fibroblasts of bone-marrow and thymic origin.

Culture of rabbit spleen cells together with antigen in plasmic dishes with an antiadhesive covering did not affect the number of AFC accumulating on the fourth day of culture. However, the addition of thymic fibroblasts to these cultures caused no increase in the number of AFC. If, however, the fibroblasts were added in the form of cells grown on coverslips, they increased the number of AFC in these cultures by 3.5-4 times.

Fibroblasts of thymic origin irradiated in a dose of 5000 R had the same effect on AFC formation in the cultures as unirradiated fibroblasts.

After the addition of stromal mechanocytes to cultures of splenic cells with antigen it was found that stromal mechanocytes of bone-marrow origin have a marked inhibitory action on AFC formation in cultures, if added during the first 48 h of culture, but had no significant effect if added later during culture.

Stromal mechanocytes of different origin, when added to cultures of nonadherent cells, exhibit an action of the same character as during association with a complete population of splenic cells. Their action is unconnected with any change in the survival rate of the cells in culture or with allogeneic combination of stromal and lymphoid cells. The differences between the action of thymic and bone-marrow fibroblasts in the presence of a deficiency of A cells show that the latter (possibly on account of the presence of splenic stromal mechanocytes among them) make the spleen cells less sensitive to the effect of mechanocytes of other hematopoietic organs.

The fibroblasts exerted their complete effect on AFC production only in the case of adhesion to the surface of the culture vessel.

The radiosensitivity of the stromal mechanocytes is reflected in a D_0 value of the order of 200 R, and consequently, irradiation in doses of 5000 R depressed the proliferative powers of fibroblasts added to the culture practically completely [2]. Proliferation of stromal fibroblasts in culture is probably not essential for the realization of their action.

The results of this investigation are evidence that certain functions of the mechanocytes are connected with the microenvironment and are directed toward the selective repression of some and stimulation of other pathways of differentiation of lymphocytes in the organs of immunogenesis.

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PROPERTIES OF T CELLS SYNTHESIZING MACROPHAGE MIGRATION INHIBITION FACTOR IN THE H-2 SYSTEM

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It was shown by means of the indirect macrophage migration inhibition test in the H-2 system, using fractionated lymphocytes, that macrophage inhibition factor (MIF) is produced by T but not by B cells. Cells producing MIF are more sensitive to the action of anti-θ-antibodies than T-killer cells. MIF formation by lymph node cells was detected at earlier periods after immunization than the cytotoxic activity of these cells. The results obtained are evidence of differences between the subpopulation of T cells synthesizing MIF and cytotoxic T lymphocytes.

KEY WORDS: inhibition of migration of macrophages; cytotoxic effect; H-2 system; T and B lymphocytes.

The macrophage migration inhibition (MMI) test in vitro is nowadays recognized to be the analog of the hypersensitivity of delayed type (HDT) test in vivo [9]. This test in mice [19], hens [14], guinea pigs [8], rabbits [15], and man [17] is a T-dependent phenomenon. Meanwhile activated B cells [18] and even cultured

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